Phytochemistry 168 (2019) 112083

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Genetic, morphological and chemical investigations reveal the genetic origin of Pompia (*C. medica tuberosa* Risso & Poiteau) – An old endemic Sardinian citrus fruit

François Luro[a,*], Grazia Viglietti[c], Elodie Marchi[a], Gilles Costantino[a], Grazia Maria Scarpa[c], Felix Tomi[b], Mathieu Paoli[b], Franck Curk[d], Patrick Ollitrault[e]

[a] UMR AGAP INRA Corse, 20230, San Giuliano, France
[b] Université de Corse - CNRS, Equipe Chimie et Biomasse, UMR SPE 6134, 20000 Ajaccio, France
[c] Dipartimento di Agraria Research Unit SACEG, University of Sassari, 07100, Sassari, Italy
[d] UMR AGAP INRA, Avenue Agropolis 34 398 Montpellier cedex 5, France
[e] UMR AGAP CIRAD Corse, 20230, San Giuliano, France

ARTICLE INFO

Keywords:
Citrus
Phylogeny
SSR markers
CPG-SM
Essential oil
Phenotype
Diversity
Genitors
Ancestral species

ABSTRACT

Citrus fruits have been introduced to the Mediterranean area from Asia for centuries and spontaneous crosses have generated several hybrid forms, some of which have had agricultural or industrial success while others have remained niche food or ornamental products, or have disappeared. Pompia (*C. medica tuberosa* Risso & Poiteau) is an old endemic citrus fruit from Sardinia of unknown genetic origin. Initial phenotypic and molecular characterizations revealed a high degree of similarity with lemon (*C. limon* (L.) Burm.) and citron (*C. medica* L.). To identify the ancestors of Pompia, 70 citrus species of the *Citrus* genus were genotyped with 36 codominant molecular markers (SSR and InDel) of nuclear and cytoplasmic genomes. Diversity analysis and allelic comparisons between each citrus species at each locus indicated that Pompia resembles lemon and limonette of Marrakech, i.e. the result of a cross between sour orange (*C. aurantium* L.) and citron, where citron was the pollinator. Two Italian citron varieties were identified as potential male parents, i.e. Diamante and Common Poncire. However, we were unable to differentiate varieties of sour oranges because varietal diversification in this horticultural group resulted from DNA sequence variations that SSR or InDel markers could not reveal. Rhob el Arsa and Poncire de Collioure were found to be two synonyms of Pompia. Pompia appeared to be equally distinct from citron, lemon and sour orange based on the overall analysis of the fruit, leaf and seed phenotype, and juice chemical composition. At the leaf level, the Pompia essential oil (EO) composition is close to that of citron whereas the zest is much closer to that of sour orange.

1. Introduction

The phylogeny of the *Citrus* genus has been extensively studied over the last 20 years by different techniques, including DNA polymorphism analysis (Nicolosi et al., 2000; Ollitrault et al., 2003; Barkley et al., 2006, García-Lor et al. 2012; Curk et al., 2016) and, more recently, genome sequencing (Wu et al., 2014, 2018; Ahmed et al., 2019). All of the findings indicate the same organization around four ancestral taxa (*C. reticulata* Blanco, *C. maxima* (Burm.) Merr., *C. medica* L. and the *Papeda* subgenus), which has given rise to many hybrids, some of which have become very important horticultural groups, such as sweet orange (*C. sinensis* (L.) Osbeck), lemon (*C. limon* (L.) Burm.), grapefruit (*C. paradisi* Macf.), sour orange (*C. aurantium* L.) and lime (*C. aurantifolia* (Christm.) Swing.). However, there are many interspecific hybrids that are cultivated only in very specific localized regions, for instance: bergamote (*C. bergamia* Risso & Poit.) is derived from a sour orange x lemon cross (Curk et al., 2016); small Chinotto sour orange (*C. myrtifolia* Raf.) of Savona (Italy); limonette of Marrakech (*C. limetta* Risso) is a popular ingredient used in the preparation of the traditional Moroccan dish *tajine*, an hybrid of citron and sour orange (Curk et al., 2016); and the Tunisian Chiiri lime, which is a probable hybrid between a 'Mexican' type lime and citron and which is used as rootstock in dry areas (Snoussi et al., 2012). All of these citrus varieties are currently becoming appreciated in an expanding niche market.

In Sardinia, the unusual citrus variety named Pompia is mainly cultivated in Siniscola (Baronia province) and transformed into honey

* Corresponding author. UMR AGAP INRA Corse, 20230, San Giuliano, France.
E-mail address: francois.luro@inra.fr (F. Luro).

https://doi.org/10.1016/j.phytochem.2019.112083
Received 27 May 2019; Received in revised form 30 July 2019; Accepted 3 August 2019
Available online 11 September 2019

fruit candy, but its origin is still unknown. The earliest records of its existence date back to the 18th century in an essay on plant and animal biodiversity of Sardinia, (Manca Dell'Arca, 1780). In a listing of citrus fruits in Sardinia, Dell'Arca cites lemons, oranges and citrons among which Pompia was classified, but without any further information. In 1837, Moris called it *Citrus medica monstruosa* and indicated that, based on a careful morphological description, *C. medica tuberosa* (Risso and Poiteau, 1818) and *C. medica* s.l. (Gallesio, 1811) were probable synonyms. Moris also reported the Italian name China citron and the vernacular name Spompia, (Chessa et al., 1994). According to Chessa et al. (1994), Pompia differs from lemon and citron with regard to some fruit axle and fruit shape aspects. Two studies based on dominant multilocus markers concluded that Pompia is a lemon x citron hybrid (Camarda et al., 2013, AFLP and RAPD markers; Mignani et al., 2015, ISSR). Camarda et al. (2013) suggested *C. limon* var. Pompia var. Nova as a new name for Pompia. However, these two studies only considered a few genotypes: citron, lemon, sour orange, grapefruit and Volkamer lemon (Mignani et al., 2015), as well as three lemon varieties and one citron variety (Camarda et al., 2013). The use of barcoding (ITS) and cytoplasmic DNA fragment sequencing of 10 citrus genotypes suggested a putative contribution of sour orange and citron in the origin of Pompia (Viglietti et al., 2019). However, definitive conclusions about the origin of Pompia require more accurate genetic diversity studies based on an extended set of codominant nuclear and cytoplasmic markers, similar to that performed to identify the origin of Corsican citron (Luro et al., 2012). Simple sequence repeat (SSR) markers have been widely used for *Citrus* genotyping and phylogenetic studies (Kijas et al., 1997; Barkley et al., 2006; Luro et al., 2008; Ollitrault et al., 2010; Biswas et al., 2012; Liu et al., 2013a, 2013b), while insertion/deletion (InDel) markers also proved efficient in discriminating the four citrus ancestral taxa (García-Lor et al., 2012; Ollitrault et al., 2014).

Essential oils (EO) are specific to varieties and taxonomic groups and are often used in comparative studies to assess the genetic diversity of a species, quantify the relationships between varieties or species, and classify unknown varieties on the basis of discriminating compounds, e.g. in mandarin (Lota et al., 2000, 2001; Fanciullino et al., 2006; Liu et al., 2013a, 2013b), kumquat (Güney et al., 2015; Sutour et al., 2016), grapefruit and citron (Luro et al., 2012). In addition, EO is the aromatic base that characterizes varieties and their processed by-products. The unique features of each citrus variety is thus described by analyzing the of EO compositions.

In the modern citrus industry, trees are multiplied by grafting on rootstocks adapted to local growing conditions. However, in Sardinia, many Pompia trees are still cultivated on their own roots, while being propagated by cuttings or sowing. Pompia seeds are polyembryonic (D'Aquino et al., 2005) and can therefore be clonally propagated by sowing. However, citrus polyembryonic seeds contain a zygotic embryo in addition to nucellar ones and can also generate seedlings with characteristics that differ from those of the mother plant (Ollitrault et al., 2003; Kepiro and Roose, 2007).

The aim of our study was to gain insight into the potential diversity of Pompia and its phylogenetic origin. Forty height Pompia trees growing at different Sardinian locations were genotyped with 52 nuclear markers (SSRs and InDels) to assess their genetic diversity. Then the Pompia phylogenetic origin was searched by comparing the Pompia genetic patterns to those of representative accessions of the four *Citrus* ancestral taxa and varieties cultivated for several centuries in the Mediterranean region, available at the INRA-CIRAD citrus Biological Resource Center (BRC). Chloroplastic SSRs (Cheng et al., 2005) and mitochondrial Indels (Froelicher et al., 2011) were also used to analyze the maternal phylogeny.

As a follow-up to the study of the genetic origins of Pompia, this study aimed to investigate the Pompia phenotype and its EO composition in leaves and fruits comparatively with its putative ancestors, so as to be able to estimate the inheritance of parental characters.

2. Results and discussion

2.1. Analysis of Pompia diversity in sardinia

By using 52 SSR and InDel markers no polymorphism was observed among Pompia samples from Sardinia, nor between them and the Tintori nursery sample. The trees studied therefore all had the same genotype and only one representative sample of the whole was used for subsequent studies. The absence of polymorphism suggests that the multiplication of trees even on their own rootstock was vegetative and that if seedlings were used they did not generate hybrids and only trees from nucellar embryos were selected. This absence of observed molecular variability does not preclude the existence of mutational polymorphisms that could induce phenotypical changes. Indeed such mutations in secondary citrus species are generally not detectable by SSR markers (Luro et al., 1995, 2000).

2.2. Nuclear genome diversity

The diversity of the *Citrus* genus was analyzed with 36 SSR and InDel markers selected among the set of 52 markers previously used to analyze the diversity of Pompia samples. Diversity in the 70 *Citrus* varieties was organized around four basic taxa which are mandarin, pummelo, citron and papeda ancestral species (Fig. 1). One cluster was represented by citron ancestral species, while also containing sweet and acid lime, lemon, limonette de Marrakech, Alemow and Pompia. All the varieties of this clade are known to be true citrons or direct citron hybrids (Luro et al., 2012; Curk et al., 2016; Ahmed et al., 2019). A second group represented pummelos that are closely linked with grapefruits and oranges; a third cluster pooled all the mandarins; the two papedas Combava and *C. micrantha* made up a fourth group. Sour orange and bergamot were linked and displayed central branching. This *Citrus* diversity structure was consistent with phylogenetic hypotheses and the findings of previous studies on the subject (García-Lor et al., 2012; Wu et al., 2014, 2018; Curk et al., 2016).

We noted that Pompia was identical to Poncire de Collioure and Rhobs el Arsa and it was genetically close to the limonette of Marrakech (genetic distance of 0.22) and slightly less to Khatta and Rangpur limes (0.31 and 0.29, respectively). Limonette of Marrakech is a [sour orange x citron] hybrid while Rangpur/Khatta limes are [mandarin x citron] hybrids (Curk et al., 2016; Ahmed et al., 2019). Other groups of varieties were genetically indistinguishable with our markers: five real lemon varieties (Eureka type), Ommeyades and Damas citrons, three sweet lime varieties, Etrog citrons, five grapefruit varieties and four sour orange varieties. Only one variety was selected from each of these groups in order not to introduce bias in the Structure representation.

Structure analysis offers indications on the composition of interspecific admixtures of modern varieties generally in accordance with phylogenetic hypotheses put forward based on WGS data (Wu et al., 2018), GBS analyses (Ahmed et al., 2019) or ancestral diagnostic marker studies (Curk et al., 2016) (Fig. 2). Indeed, mandarins, pummelos, citrons and papedas emerge as representative of four ancestral constitutives genomes, i.e. *C. reticulata*, *C. maxima*, *C. medica* and C. *micrantha*, respectively. Fuzhu and King appeared to have introgressed a small portion of the *C. maxima* genome, as previously observed by Wu et al. (2014) and Oueslati et al. (2016) based on DNA sequence data. All pummelos appeared has pure representatives of *C. maxima*, except for Hog pummelo with 2/3 *C. maxima* and 1/3 *C. reticulata* contributions, similar to the pattern in grapefruit. The sweet orange genome displayed *C. reticulata* and *C. maxima* admixtures (55% and 45% respectively). Rough lemon, Khatta lime and Volkamer lemon appeared to have close to half *C. medica* and half *C. reticulata* contributions, while Adam's apple, Nestour Lime and Alemow displayed balanced contributions of *C. medica* and *C. micrantha*. DNA sequencing based genome analyses, however, revealed some minor differences. Pummelo genome introgressions in mandarin are infrequent, whereas they have been

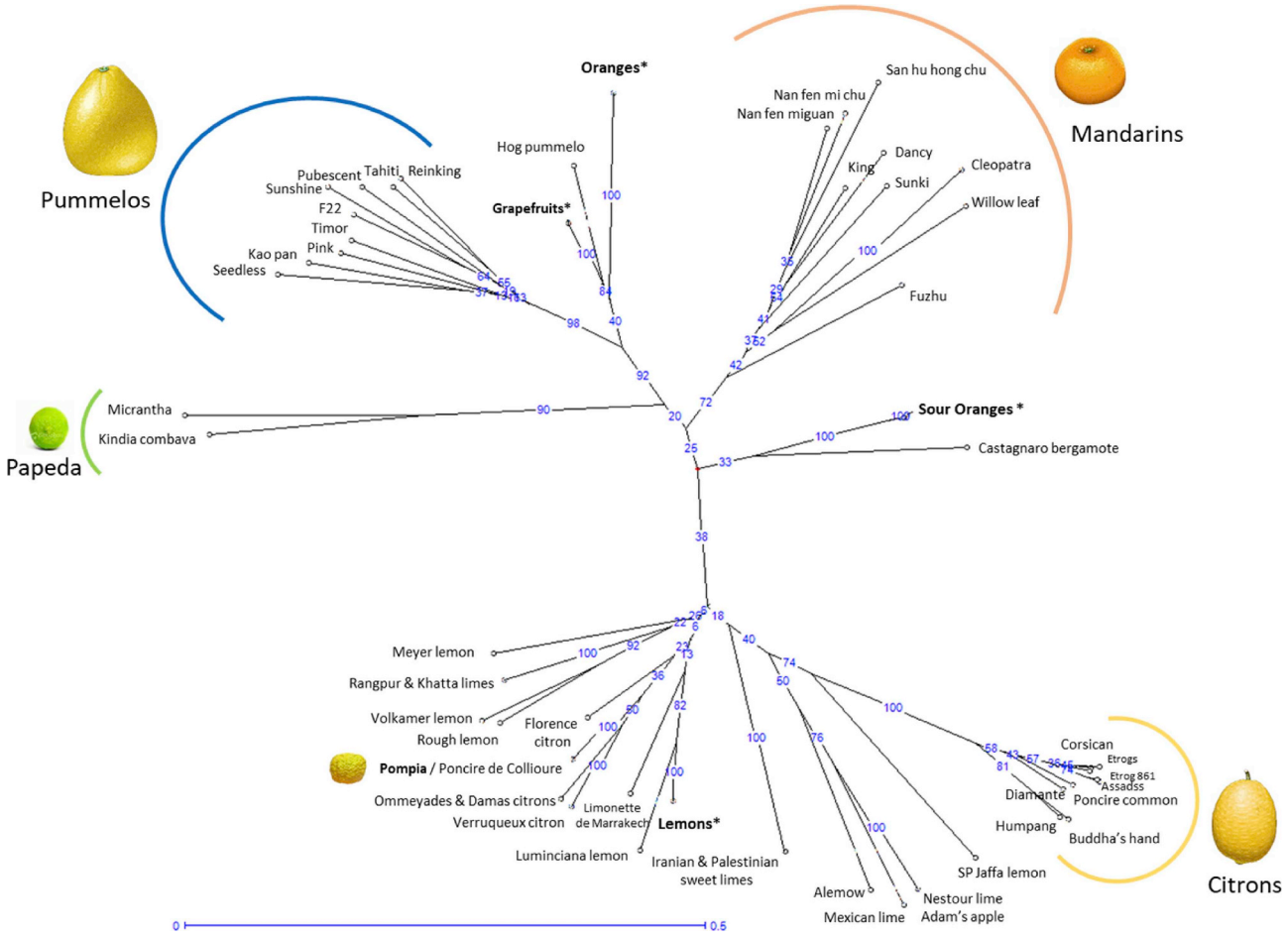

Fig. 1. Nuclear diversity and relationships of 70 citrus varieties investigated with 36 SSR and InDel markers using simple matching similarity index and NJ tree portioning. Lemon, orange, grapefruit and sour orange were represented by a single genotype because of absence of infragroup polymorphism. Values on the branches correspond to 500 bootstraps. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

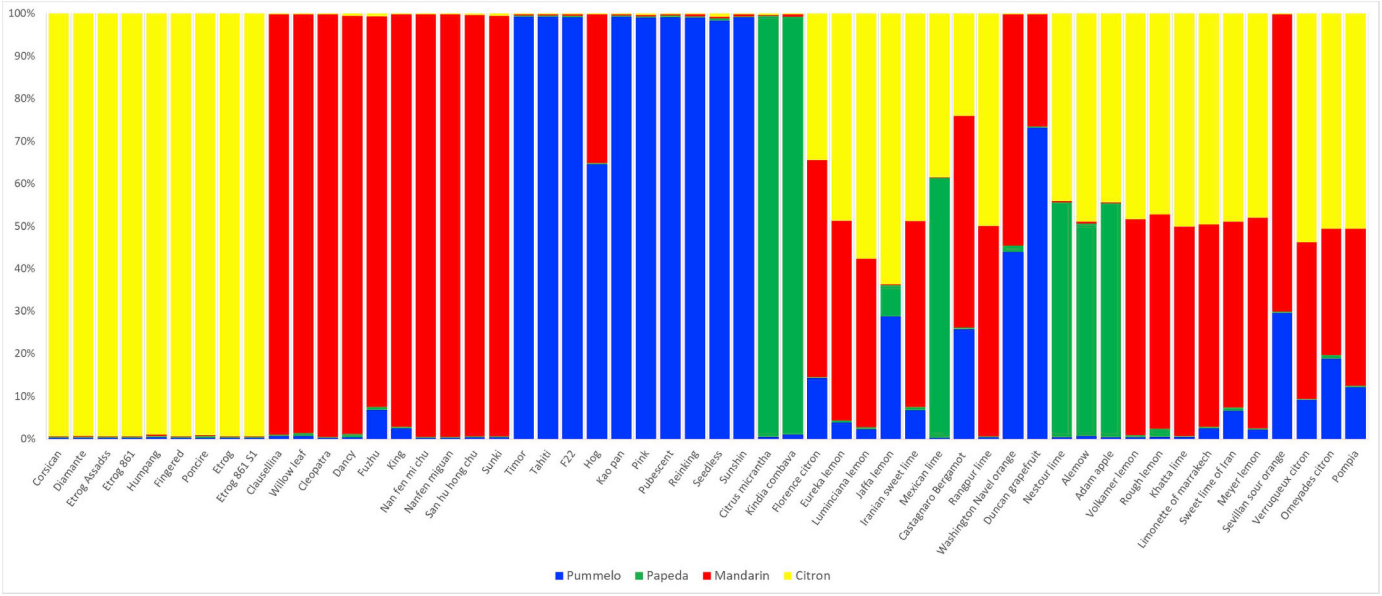

Fig. 2. Contributions (%) of the four ancestral taxa to the 23 citrus varieties, including Pompia. Structure software analyses using 36 InDel and SSR markers (average values for 10 runs with k = 4). (blue: *C. maxima*; red: *C. reticulata*; green: Papeda; yellow: *C. medica*). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

observed in all modern cultivated mandarin varieties, with the exception of Sunki and Cleopatra varieties (Oueslati et al., 2016; Wu et al., 2018). The present study did not detect any pummelo genome introgressions in edible mandarins. This could be due to the relatively low number of used markers compared to the thousands of SNPs detected by WGS and GBS. In several interspecific hybrids, the proportion of pummelo alleles also appeared to be slightly under-represented, compared to findings based on WGS data (Wu et al., 2018), GBS analyses (Ahmed et al., 2019) or ancestral diagnostic marker studies (Curk et al., 2016). Despite this minor divergence, the present Structure analysis based on SSR and InDel markers consistently identified the main contributions of ancestral taxa to the analyzed germplasm.

Regarding Pompia, the analysis demonstrated the complex interspecific hybrid nature of its origins, i.e. 50% *C. medica*, 35% *C. reticulata* and 15% *C. maxima* admixture. Likely the most relevant hypothesis is that it resulted from hybridization between a citron and a variety with and interspecific *C. maxima* and *C. reticulate* admixture, but at this stage we cannot preclude the hypothesis of hybridization between lemons or sweet limes.

2.3. The parental origin of Pompia

2.3.1. Allelic nuclear genome composition

We first estimated the LAP index for all the studied genotypes to select the different putative parental candidates of Pompia. Only genotypes with an index greater than or equal to 95%, were selected. Potential genotyping errors were taken into account in the genotype selection by not selecting only those with 100% loci sharing at least one Pompia allele. Sixteen varieties as potential parents have been selected (Table 1). The LGP index estimating the proportion of loci carrying the two Pompia alleles was then calculated for all paired combinations of these 16 genotypes (Table 1). Of the 16 genotypes, 10 had a 100% LAP index, including sour orange, four citrons, three [mandarin x citron] hybrids, one [sour orange x lemon] hybrid (Florence citron), one [sour orange x citron] hybrid (Limonette of Marrakech) and a citron hybrid with an unknown maternal parent (Verruqueux citron). Lemon is often considered to be a possible parent of Pompia, but 5% of the markers did not have at least one common allele with Pompia. Only two combinations corresponded perfectly to the expected allelic complementation and could generate Pompia by crossing: [sour orange - Diamante citron] or [sour orange - Common Poncire citron]. For 10% of the loci, the sour orange – lemon combination did not match the Pompia genotype. This proportion increased to 45% for the [citron – lemon] combination proposed by Camarda et al. (2013) based on a dominant marker (ISS) analysis.

Pompia is therefore likely the result of a similar *C. aurantium x C. medica* interspecific hybridization as lemon. Mignani et al. (2015) have already observed the affinity between Pompia and lemon, on the basis of an analysis with dominant AFLP and RAPD markers and some co-dominant SCAR markers. This study also proposed a possible parent-offspring relationship between Etrog citron and Pompia. However the use of dominant multilocus markers did not allow a complete allelic reconstruction of each locus.

Poncire commun and Diamante citrons both originated from Italy. Common Poncire may have generated the 'Corsican' variety by self-fertilization (Luro et al., 2012). These two citrons were commonly cropped in Italy and Sardinia. Sour orange—introduced by the Moors in the 8th century (Calabrese, 1990)—was present in many Mediterranean regions and used for ornamental, food and perfumery (candied fruits, jams, floral water, EO) purposes. Spontaneous sour orange x citron hybrids were likely quite frequent in the Mediterranean Basin as the trees of both species were grown in the same places and often multiplied by seedlings.

2.3.2. Genetic diversity inferred by maternally inherited markers

Six genetic profiles (cytotypes) were obtained on the basis of the polymorphism of chloroplast and mitochondrial markers (Fig. 3). Mandarins were divided into two groups, one corresponding to edible varieties and the other to rootstock varieties associated with the mandarin x citron hybrids revealed by the nuclear analysis. The citron group only included citron varieties but no nuclear admixture genotypes. The pummelo cytotype was shared with sweet orange, grapefruit, sweet lime, Meyer and Jaffa lemons. All of these observations were in agreement with the results previously obtained by Curk et al. (2016). The sour orange cytotype was the same as that of lemon, bergamot, limonette of Marrakech, and some citrus fruits mistakenly named citrons and Pompia. The nuclear genome and cytoplasmic genome profiles suggest that Jaffa lemon arose from a pummelo x citron cross, while Verruqueux citron and Pompia likely resulted from hybridizations between sour orange and citron, with citron being the pollinator in all cases. These observations complete the list of examples of previously described offspring where citron was systematically identified as the male parent, (Luro et al., 2012; Curk et al., 2016; Wu et al., 2018). Are there any inhibitions to maternal inheritance of citron in

Table 1
Proportion (%) of loci carrying at least 1 Pompia's allele (LAP) and proportion (%) of complementary combinations of each pairs of candidate reproducing the Pompia genotype. Among the initial 70 citrus varieties only the 16 genotypes with a LAP index superior to 90% were considered.

Id	Citrus genotype	LAP[b]	Proportion of loci with both Pompia alleles for each genotype pair (%)[a]															
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	Sour orange	100	5															
2	Corsican citron	100	95	10														
3	Diamante citron	100	100	10	10													
4	Etrog Assadss citron	100	95	10	10	10												
5	Poncire commun citron	100	100	10	10	10	10											
6	Verruqueux citron	100	90	65	65	65	65	45										
7	Damas citron	95	90	65	65	65	65	70	65									
8	Etrog citron	95	90	10	10	10	10	55	65	10								
9	Etrog 861 citron	95	90	10	10	10	10	55	65	10	10							
10	Florence citron	100	65	85	85	85	85	90	90	85	85	55						
11	Eureka lemon	95	90	55	55	55	55	65	65	55	50	80	50					
12	Rangpur lime	100	85	60	60	60	60	70	80	55	55	75	75	45				
13	Volkamer lemon	95	75	60	60	60	60	70	75	60	60	75	65	70	40			
14	Rough lemon	95	85	60	60	60	60	65	70	55	55	75	60	65	55	45		
15	Khatta lime	100	85	60	60	60	60	70	80	55	55	75	75	45	65	65	45	
16	Limonette of Marrakech	100	90	70	70	70	70	75	80	65	65	80	75	65	80	70	65	60

[a] The 2 alleles of Pompia are carried by both genotypes.
[b] LAP: Proportion of loci with at least one allele of Pompia.

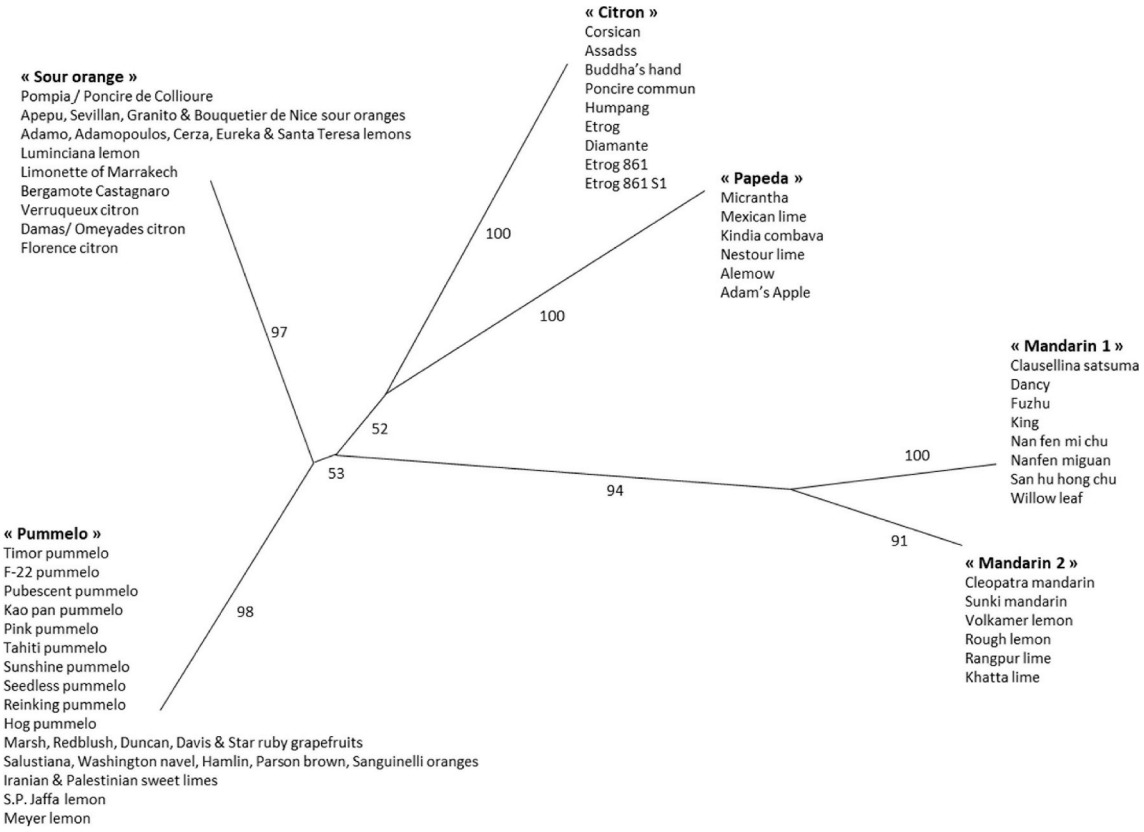

Fig. 3. Cytoplasmic profile assignation of the 70 citrus varieties. A neighbor joining tree established from thre mitochondrial InDel markers and three chloroplastic SSR markers based on the genetic distances of each citrus pair calculated using the simple matching dissimilarity index. Values on the branches represent the 500 bootstrap reiterations.

interspecific crosses or is it related to cleistogamy, which is highly predominant in citrons? The latter hypothesis seems more likely because we successfully obtained interspecific citron hybrids in carefully controlled cross-fertilization experiments. Note that the Corsican citron variety derives from self-fertilization of Common Poncire citron (Luro et al., 2012). We conclude that Pompia, lemon, limonette of Marrakech and Verruqueux citron all result from similar hybridizations between sour orange as female parent and citron as pollinator, even though we were unable to clearly identify the parental varieties. Moreover, for Pompia we suggest that the male parent is an Italian citron variety such as Diamante or Common Poncire.

3. Pompia phenotype compared to that of its parents and lemon

The characters that were found to bring Pompia significantly (Fisher's test) closer to sour orange were: fruit shape (flattened at the poles), empty fruit axis (Fig. 4), perceptible wrinkles at the seed surface, seed polyembryony (highly > 2.5) and large leaf size (Supplementary files 1 and 2). Common characters between Pompia and citron were: the presence of areola at the fruit apex, peel color (yellow) (Fig. 4), juice sugar content and acidity and the presence of a curved beak at the end of the seed formed by chalaza integuments (Supplemental files 1 and 2). The fruit segment number was the only character for which Pompia presented the highest values relative to the three other species.

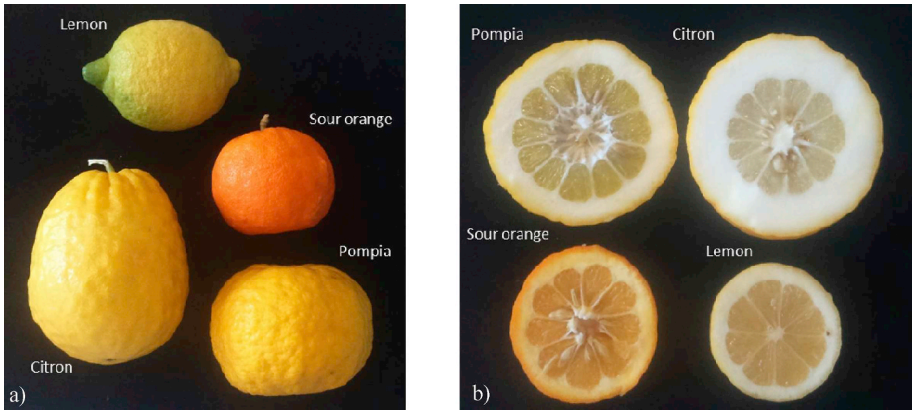

Fig. 4. Fruit of Pompia, citron, sour orange and lemon: a) fruit side, b) inside view of the fruit cut along the equatorial plane. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

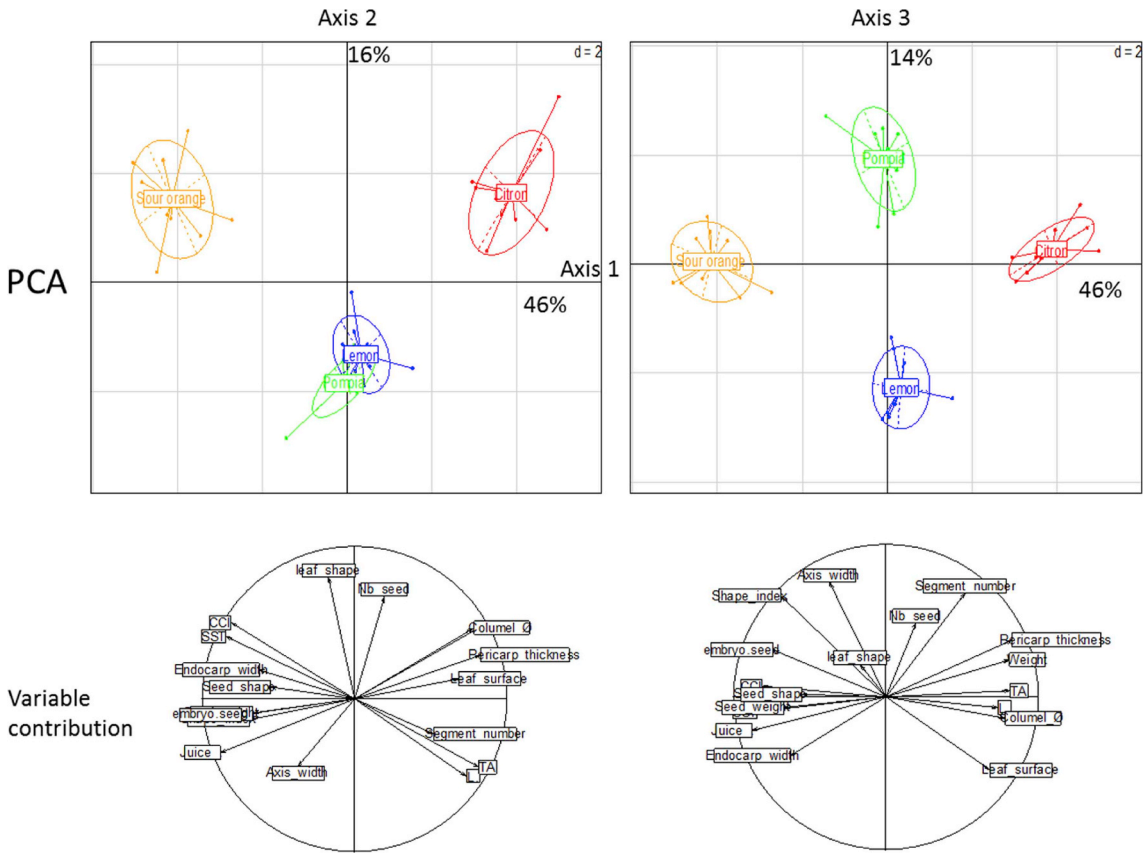

Fig. 5. PCA of the diversity revealed by 18 fruit, leaf and seed phenotypic characters among Pompia, citron, sour orange and lemon samples and the contribution of the characters to the dispersion on the 1, 2 and 3 axes. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

PCA was performed to represent the phenotypic diversity of the four varieties and the variables that differentiate them (Fig. 5). Correlations between variables were previously measured so as to only include independent variable in the PCA. The variables taken into consideration were: fruit weight, fruit shape, peel color (the three indices L *, a * and b *), percentage of pericarp thickness relative to the fruit diameter, recess axis size, columella diameter, number of fruit segments, number of seeds per fruit, percentage juice, juice acidity and sugar content, leaf shape and surface, seed shape and weight and number of embryos for seed.

The phenotypic differentiation between sour orange and citron was supported by fruit weight and height, pericarp thickness, axis width, juiciness, TSS, seed weight and length and embryony on axis 1 as representing 46% of the total variability, with very similar intermediate positions for lemon and Pompia. It could be concluded that the phenotype of the two lemon and Pompia hybrids was largely intermediate between the two parents. The considered variables thus had additive inheritance. The second axis differentiated lemon and Pompia on one side and their two parents on the other, while the third axis clearly distinguished lemon from Pompia, with their two parents having an intermediate position. The contribution of the variables to axis 3 provided information on the distinguishing features of lemon and Pompia, i.e. the central axis diameter, columella presence, fruit weight and shape. Considering the interspecific origin of sour orange (*C. maxima x C. reticulata*), while most citrons are highly homozygous (Luro et al., 2012; Curk et al., 2016; Wu et al., 2018), it is likely that the differentiation between lemon and Pompia primarily resulted from the segregation between *C. maxima* and *C. reticulata* genomes in the two ovules that generate lemon and Pompia.

Based on the phenotypic characterization of Pompia conducted in Sardinia (D'Aquino et al., 2005), most of the characters of Pompia grown in Corsica were very close to those of Pompia grown in Sardinia. Trees grown in Sardinia that were studied (D'Aquino et al., 2005; Mignani et al., 2004) were generally located in commercial orchards, while those studied in Corsica were located in a collection close to other citrus species and varieties. The same rootstock, i.e. sour orange, was used in both cases. There were, however, some differences: in Corsica the fruits were smaller, slightly more juicy and sweet, and slightly less acidic. This may have been due to changes in the growing conditions, cultural technology or the environment. In the collection, the main objective is to preserve the trees and not to promote high fruit yields. Consequently, annual pruning and fertilization are not focused on the same requirements: nitrogen fertilization actually tends to increase the fruit size. The greatest variability between the two sites was observed with regard to the number of seeds per fruit, with an average of 10 in Sardinia compared to 20 in Corsica. This difference could be explained by variations in pollen pressure during flowering between a monovarietal orchard geared towards fruit production as in Sardinia and the Corsican collection which includes many different species in the vicinity of the Pompia trees.

4. Pompia essential oil composition compared to that of its parents and lemon

The percentages of compounds detected in EO in the analyzed citrus leaves and fruits are listed in Supplementary file 3. Since repeat EO extractions were not carried out for the four citrus profile comparisons, we focused exclusively on the majority compounds: > 3% leaf essential oil and > 1% peel EO.

There were numerous quantitative and qualitative (presence/absence) differences between the four citrus species. When only considering the 15 major compounds (Fig. 6), the EO profile of Pompia

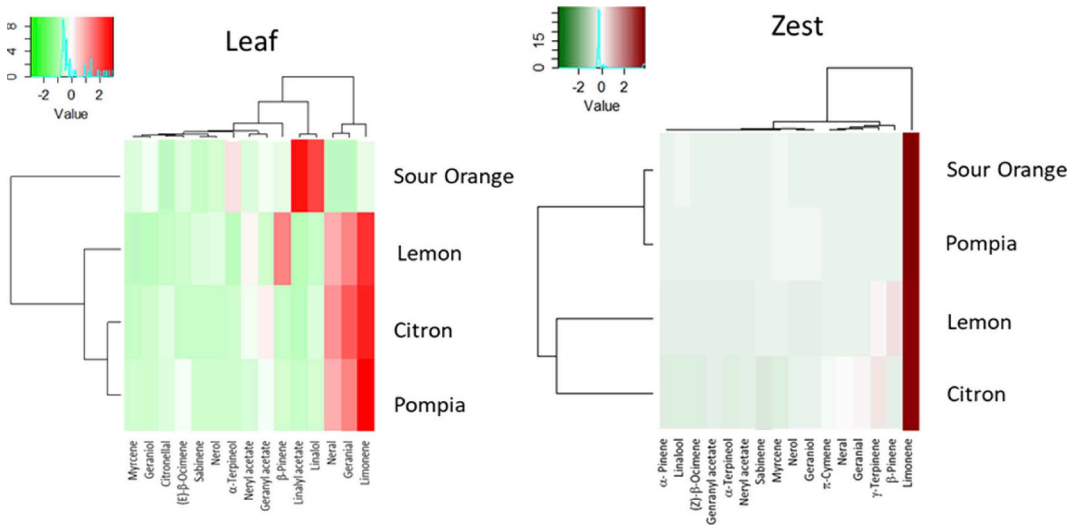

Fig. 6. Heat map of relationships between citrus genotypes related to the contribution of the major different leaf and zest EO compounds (color range represents the variance level of the quantitative character with respect to the average). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

leaves was close to that of citron, and only the percentage of neryl acetate in Pompia was closer to that of sour orange. A profile emerged with about 30% limonene, 20% geranial, 15% neral (major compounds) for Pompia and citron, while lemon had a profile that differed especially in terms of the high percentage of β-pinene (25% limonene, 15% geranial, 15% β-pinene and 10% neral). The sour orange chemotype was completely different: 35% linalyl acetate; 25% linalool, and 10% α-terpineol. In Pompia EO, only limonene and (E)-β-ocimene had higher rates than those of the other three citrus species.

Limonene was found to be the major constituent of zest EO (50–93.5%). The number of compounds detected was lower as compared to the number in leaf EO (39 versus 58, respectively). Based on the 12 major compounds, it was found that Pompia EO had a very high limonene content (> 90%), as is the case in sour orange, while the limonene percentage was lower, i.e. around 50% in citron and lemon. Apart from limonene, the presence and proportion of geraniol and nerol were close to the levels of citron, while the two compounds were absent in sour orange and lemon. Lemon zest EO stood out from that of the other three varieties, mainly with regard to the elevated β-pinene (12%) and sabinene content.

The EO main components in Pompia, citron and lemon fruit peel and leaves of were similar to those observed in 1997–1998 (Lota et al., 1999, 2002) in a study conducted with the same varieties from the INRA-CIRAD citrus collection at San Giuliano. This suggested a certain degree stability over time regardless of the tree age. However, different results were reported for the EO composition of Pompia fruit peel in Sardinia: limonene content of 77.5% (Fenu et al., 2010), 93.3% (Camarda et al., 2013) and 77.4% (Flamini et al., 2019) or 90% (Rosa et al., 2019).

Petretto et al. (2015), by a different process of extraction (saturation in the head space), also obtained a rich Pompia limonene profile (94.1%), which was close to that of sour orange. The sour orange profile differed from that of Pompia, as there was a relatively high linalool concentration (about 5% versus 0.1%. The citron profile in the present study differed from that of Petretto et al. (2015) by the lower proportion of limonene (50% versus 71%) and higher nerol, neral, geraniol, geranial, neryl acetate and geranyl acetate levels (1.3–7.4% versus < 1%). These variations may have been due to the process of EO extraction or a cultivar effect, i.e. Diamante in their study and Common Poncire in the present study. The variability in the EO composition between these two cultivars has already been highlighted (Luro et al., 2012).

The Pompia leaf EO profile was close to that of citron, with average amount of the major components, including as limonene (36%) neral (15.6%), geranial (20.4%) and geranyl acetate (5.0%). The leaf chemical profile was close to that reported by Flamini et al. (2019) and Fancello (2016), where the amounts of the same components were in 28.6%–28%, 18.8%–18.9%, 24.4%–23.4% and 3.9%–2.1%, respectively. Differences noted in the leaf and fruit EO compositions demonstrated tissue-dependent differentiated inheritance as a result of the different regulation of the synthesis of volatile compounds between the flavedo and leaves. The predominance (over 90%) of limonene in the Pompia and sour orange zest EO should also be considered. In a proportional relation, it is evident that in this case the other molecules were limited and their quantitative variations could be under- or overestimated. In the leaf EO, limonene represented at most 1/3 of the total amount of compounds, increasing the proportion of the minor components. In our study, the high level of limonene (as in sour orange) combined with the presence of geraniol and nerol (as in citron) contributed to the aromatic specificity of Pompia.

5. Conclusion

The Pompia admixture origin was determined by studying the polymorphism of nuclear genome DNA markers, while chloroplast and mitochondrial markers revealed the maternal phylogeny. Like lemons and limonette of Marrakech, Pompia was found to be derived from a citron x sour orange cross, with citron as male parent. Although the SSR or InDel markers did not discriminate the sour orange varieties, they revealed polymorphism among citrons and suggested that the Italian Diamante and Common Poncire varieties could be the best Pompia male parent candidates. Rhobs el Arsa and Poncire de Collioure were found to be two synonyms of Pompia. The morphological and biochemical characterization corroborated the parental relationship of Pompia with these parent species, but also highlighted the difficulty of elucidating phylogeny based only on phenotypic characters. Although the Pompia zest EO composition was relatively close to that of sour orange, it had a very specific aroma. To obtain Pompia by crossbreeding, it would be necessary to obtain further confirmation on the varietal identity of the sour orange and citron parents. Genotyping by sequencing (GBS) or NGS could provide useful information in this respect.

6. Experimental

6.1. Genetic analysis

6.1.1. Plant material

Pompia plant sampling was carried out at different sites in Sardinia: 48 samples were collected in different localities: 2 from Milis, 1 from Bitti, 8 from Oliena and 37 from Siniscola (6 orchards) (Supplemental File 4). Sampling sites were chosen to represent the geographic distribution of Pompia in Sardinia. Sampled trees were located in private gardens and differed markedly in terms of age and growing conditions. Some trees were grafted on sour orange rootstock while others were not grafted. One Pompia accession originating from the nursery of Oscar Tintori in Pescia (Toscany, Italy) was also included as an external geographical control.

To analyze the diversity and detect putative parents of Pompia, 70 citrus varieties were chosen to represent the genetic diversity of *Citrus* (Supplemental File 5). All of the studied accessions are maintained at the Citrus Biological Resources Center (BRC) INRA-CIRAD at San Giuliano, Corsica (France) (Luro et al., 2018). Varietal number for the ancestral species are: 10 pummelos, 10 mandarins, 9 citrons and 2 papedas. In addition to the four ancestral species, 5 varieties of each secondary species were chosen: sweet orange (*C. sinensis* (L.) Osb.), sour orange (*C. aurantium* L.), lemon (*C. limon* (L.) Burm.) and grapefruit (*C. paradisi* Macf.). Nineteen supplementary citrus varieties, including acid lime (*C. aurantifolia* (Christm.) Swing. and Castagnaro bergamot (*C. bergamia* Risso & Poit.), were added and some of them have long been present in the Mediterranean zone. Mexican lime (*C. aurantifolia* (Christm.) Swing.), a direct product of an ancient cross between *C. micrantha* Wester and *C. medica* (Nicolosi et al., 2000), was also included since it is cultivated in some southern Mediteranean Basin dryland areas (Snoussi et al., 2012) and considered as the progenitor of several lime varieties (Snoussi et al., 2012; Curk et al., 2016).

6.1.2. Genotyping by DNA markers

DNA extraction, PCR amplification, banding pattern electrophoresis and marker scoring were conducted according to the method described by Luro et al. (2008).

Molecular markers: the maternal origin of Pompia was studied using six markers of chloroplastic and mithochondrial genomes: Ccmp5, Ccmp6 (Weising and Gardner, 1999) and Ntcp9 (Bryan et al., 1999) as plastidial markers and three mitochondrial (mtDNA) markers (nad 7 1/2, 4/3 and nad 2 rrn 5/18-1) developed by Froelicher et al. (2011). For the nuclear diversity study, 52 SSR and InDel markers were chosen based on their chromosomal location with broad genome dispersion to verify the polymorphism of Pompia samples from Sardaigna (Supplemental File 6). Subsequently, among these 52 markers 36 markers were selected according to the quality of the amplification profiles and according to the heterozygosity of the Pompia locus, to perform the following diversity analysis.

6.1.3. DNA molecular data analysis

Genetic relationships between the different varieties were analyzed with DARwin software (Perrier et al., 2003) using the weighted neighbor joining method, based on the 'Simple matching' similarity index, which took into account the percentage of common alleles between two citrus samples divided by the total number of observed alleles.

The genomic structure of citrus hybrids (including Pompia) was inferred with STRUCTURE version 2.3.4 (Pritchard Lab, 2014), which implements a model-based clustering method using genotype data (Pritchard et al., 2000; Falush et al., 2003). We opted for the linkage model with correlated allele frequencies. STRUCTURE was run 10 times with 50,000 burn-in steps followed by 50,000 Monte Carlo Markov Chain (MCMC) repetitions. For K = 4, the output clusters of 10 independent STRUCTURE runs were permuted and aligned, and the average frequency and standard error of the contribution of each basic population were estimated.

Two indices were calculated to infer the most probable Pompia parents: first was the proportion of loci for each genotype sharing at least one Pompia allele (LAP); and the second index was calculated for each genotype pair and represented the proportion of loci in each pair of genotypes sharing the two Pompia alleles (LGP), (one allele by genotype sharing). A pair of genotypes was considered as possible parents of Pompia when both genotypes include the Pompia genotype. The LAP index was first used to reduce the number of candidates before calculating the second index (LGP) for all paired combinations of the selected candidates. The efficiency of Pompia parent detection depended on the species discrimination power of the markers used.

6.2. Phenotyping

6.2.1. Plant material

Trees of the four species used in the evaluation, i.e. *C. limon* var. Santa Teresa (ICVN010626), *C. medica* var. Common Poncire (ICVN010701), *C. aurantium* var. Sevillan (ICVN010033), and *C. aurantium* x *C. medica* var. Pompia (ICVN0110244), are grown in the BRC INRA-CIRAD citrus collection at San Giuliano (Corsica) (latitude 42°27′N - longitude 9°32′E) under the same conditions (watering, fertilization, size, pest control treatments). All trees grafted on *C. volkameriana* were more than 15 years old.

For phenotyping, 15 fruits and leaves per accession were collected randomly at eye level on the periphery of the three trees on different branches, provided that illness, injury or deficiency symptoms were absent. The fruits were collected in January at full maturity for all species.

6.2.2. Phenotypic characters of leaves, seeds and fruits

The observed characters were: fruit weight (g), fruit diameter and height (mm), fruit shape (height/width), peel color (Cartesian coordinates L *, a * and b*), pericarp (exocarp + albedo) and endocarp (mm) thickness, fruit axis and columel diameter (mm), number of sectors, juice content (g/100 g of fruit), total soluble solids (TSS) expressed in °Brix and titratable acidity (TA) expressed as mg of citric acid/100 mg of juice, seed number per fruit, seed weight (mg), seed shape (length/width), leaf blade surface (cm^2) and leaf shape (length/width). Qualitative characters were also evaluated such as the presence of an integumentary break at the seed chalazae level, the presence of areola at the fruit apex and the presence of wrinkles on the seed surface. In this case, the fruit tissue weight and thickness were measured with a precision balance and caliper, respectively. The proportion (percentage by weight) of juice was measured after manual pressure by extracting the juice using a juicer. The juice was filtered through a 1 mm mesh sieve before weighing. TA measurement was performed using a Mettler Toledo DL50 titrator. TSS was estimated using an automatic RFM710 refractometer (Bellingham + Stanley Ltd.).

The peel color was measured based on the L* a* b* indices of CIE 1976 color space (CIELab) through a Minolta CR300 colorimeter. The staining parameter CCI (Citrus Color Index) created to measure peel color changes (degreening) in clementines (Jiménez-Cuesta et al., 1981) is a combination of the three indices, ICC = (1000 x a*)/(L* x b*). Three measurements were done at three different points on the equatorial axis of each fruit. The average of the three values represented the fruit color. The seed and leaf length and width, and the leaf area were estimated using ImageJ software based on a photo of the organs and comparing it with a reference of 20 mm and 4 cm^2, respectively.

6.2.3. Analysis of essential oil (EO) composition

6.2.3.1. Essential oil (EO) extraction. From the same four species chosen for phenotypic comparison, ripe fruits and leaves at their maximum development were also gathered in January from the periphery of three

trees for EO extraction. Leaf and fruit peel EO were extracted by hydrodistillation using a Clevenger type apparatus. 200 g of the fruit peel or 300 g of leaves were placed in a 2 l glass bowl. The citrus material set has been brought to and kept at a boil for 3 h (Lota et al., 2000). The essential oil was recovered and separated from the hydrolat via the density difference.

6.2.3.2. EO components separation and detection. .Gas chromatography (GC): analyses were performed on a Perkin Elmer Clarus 500 gas chromatograph (FID) equipped with 2 fused silica gel capillary columns (50 m, 22 mm id, film thickness 0.25 μm), BP-1 (polydimethylsiloxane) and BP-20 (polyethylene glycol). The oven temperature was programmed from 60 to 220°C at 2°C/min and then held isothermal at 220°C for 20 minutes, with injector temperature 250°C, detector temperature 250°C, carrier gas hydrogen (1.0 mL/min), and split 1/60. The relative proportions of the oil constituents were expressed as percentages obtained by peak area normalization, without using correcting factors. RIs were determined relative to the retention times of a series of *n*-alkanes (C7-C28) with linear interpolation ('Target Compounds" software of Perkin Elmer). *Gas chromatography coupled with mass spectrometry (GC/MS):* The EOs were analyzed with a Perkin Elmer TurboMass detector (quadrupole), directly coupled to a Perkin Elmer Autosystem XL, equipped with a fused silica gel capillary column (50 m, 0.22 mm id, film thickness 0.25 μm), (BP-1 polydimethylsiloxane). Carrier gas, helium at 0.8 mL/min; split, 1/75; injection volume, 0.5 μL; injector temperature, 250°C; oven temperature programmed from 60 to 220°C at 2°C/min and then held isothermal (20 min); ion source temperature, 250°C; energy ionization, 70 eV; electron ionization mass spectra were acquired over the mass range 40-400 Da. *Identification of components*: identification of the components was based: (a) on comparison of their GC retention indices (RI) on polar and apolar columns, determined relative to the retention times of a series of *n*-alkanes with linear interpolation with those of authentic compounds and literature data (Davies, 1990; Joulain and König, 1998); (b) on computer matching against NIST commercial mass spectral library (National Institute of Standards and Technology, 1999) and by comparison of spectra with literature data (Joulain and König, 1998; König et al., 2001; Adams, 2007).

6.2.4. Analysis method of phenotype data

Phenotypic data were analyzed using R software and the basic packages for calculating means and standard deviations. The Agricolae package for variance analysis and Fisher's least significant difference (LSD) test at an α risk of 0.05, and Pearson's base package, PCA correlations carried out with the Ade 4 package for the principal component analysis (PCA). For PCA, the values of each variable were centered and reduced to obtain variations of the same size among variables. Heat maps were constructed using R software with the g plots package to analyze the EO data and determine the relationships between varieties and components contributing to this diversity.

Acknowledgments

This research was funded by "Programme de Coopération Italie-France "Maritime" 2014-2020 - I appel pour projects simples et stratégiques Thématiques et territoriaux pour les axes prioritaires 1-2-3 - Project "MARE DI AGRUMI" - Axe 1 – CUP C26D16007240007"INTERREG is an UE funding corresponding to a bilatteral collobaration program between France and Italy with including regions close to the sea on the north part of Mediterranean sea called Maritimo, the name of th project was Mare di Agrumi in italian and Mer d'Agrumes in french (citrus sea).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.phytochem.2019.112083.

References

Adams, 2007. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. In: 4th ed. Allured Publ. Corp., Carol Stream.
Ahmed, D., Comte, A., Curk, F., Costantino, G., Luro, F., Dereeper, A., Mournet, P., Froelicher, Y., Ollitrault, P., 2019. Genotyping by sequencing can reveal the complex mosaic genomes in gene pools resulting from reticulate evolution; a case study in diploid and polyploid citrus. Ann. Bot. https://doi.org/10.1093/aob/mcz029.
Barkley, N.A., Roose, M.L., Krueger, R.R., Federici, C.T., 2006. Assessing genetic diversity and population structure in a citrus germplasm collection utilizing simple sequence repeat markers (SSRs). Theor. Appl. Genet. 112, 1519–1531.
Biswas, M.K., Chai, L., Mayer, C., Xu, Q., Guo, W., Deng, X., 2012. Exploiting BAC-end sequences for the mining, characterization and utility of new short sequences repeat (SSR) markers in Citrus. Mol. Biol. Rep. 39, 5373–5386.
Bryan, G.J., McNicoll, J., Ramsay, G., Meyer, R.C., De Jong, W.S., 1999. Polymorphic simple sequence repeat markers in chloroplast genomes of Solanaceous plants. Theor. Appl. Genet. 99, 859–867.
Calabrese, F., 1990. La favolosa storia degli agrumi. Agricoltura 208, 83–128.
Camarda, I., Mazzola, P., Brunu, A., Fenu, G., Lombardo, G., Palla, F., 2013. Un agrume nella storia della Sardegna: *Citrus limon* var. Pompia Camarda var. Nova. Quad. Bot. Amb. Appl. 24, 109–118.
Chessa, I., Mulas, M., Pala, M., 1994. Gli agrumi. In: Agabbio, M. (Ed.), Patrimonio Genetico di Vecchie Specie Arboree da Frutto: Le vecchie varietà della Sardegna Carlo Delfino Editore, pp. 339–360 Sassari.
Cheng, Y., de Vicente, M.C., Meng, H., Guo, W., Tao, N., Deng, X., 2005. A set of primers for analyzing chloroplast DNA diversity in Citrus and related genera. Tree Physiol. 25, 661–672.
Curk, F., Ollitrault, F., Garcia-Lor, A., Luro, F., Navarro, L., Ollitrault, P., 2016. Phylogenetic origin of limes and lemons revealed by cytoplasmic and nuclear markers. Ann. Bot. 117, 565–583. https://doi.org/10.1093/aob/mcw005.
D'Aquino, S., Fronteddu, F., Usai, M., Palma, A., 2005. Qualitative and physiological properties of 'Pompia', a citron-like fruit. Plant Genet. Resour. Newsl. 143, 40–45.
Davies, 1990. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20M phases. J. Chromatogr. 503, 1–24.
Falush, D., Stephens, M., Pritchard, J.K., 2003. Inference of population structure using Multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164, 1567–1587.
Fancello, et al., 2016. Chemical characterization, antioxidant capacity and antimicrobial activity against food related microorganisms of *Citrus limon* var. *pompia* leaf essential oil. Food Sci. Technol. 69, 579–585.
Fanciullino, A.L., Tomi, F., Luro, F., Desjobert, J.M., Casanova, J., 2006. Chemical variability of peel and leaf oils of mandarins. Flavour Fragrance J. 21, 359–367.
Fenu, G., Carai, A., Foddai, M., Azara, E., Careddu, S., Usai, M., 2010. Composition and seasonal variation of *Citrus monstruosa* essential oil from Sardinia. Int. J. Essent. Oil Therap. 4, 23–25.
Flamini, G., Pistelli, L., Nardoni, S., Ebani, V.V., Zinnai, A., Mancianti, F., Ascrizzi, R., Pistelli, L., 2019. Essential oil composition and biological activity of "Pompia", a Sardinian citrus ecotype. Molecules 24, 908. https://doi.org/10.3390/molecules24050908.
Froelicher, Y., Mouhaya, W., Bassene, J.B., Costantino, G., Kamiri, M., Luro, F., Morillon, R., Ollitrault, P., 2011. New universal mitochondrial PCR markers reveal new information on maternal citrus phylogeny. Tree Genet. Genomes 7, 49–61.
Gallesio, G., 1811. Traité du Citrus. Louis Fantin, Paris.
García-Lor, A., Luro, F., Navarro, L., Ollitrault, P., 2012. Comparative use of InDel and SSR markers in deciphering the interspecific structure of cultivated citrus genetic diversity: a perspective for genetic association studies. Mol. Genet. Genom. 287, 77–94. https://doi.org/10.1007//s00438-011-0658-4.
Güney, M., Oz, A.T., Kafkas, E., 2015. Comparison of lipids, fatty acids and volatile compounds of various kumquat species using HS/GC/MS/FID techniques. J. Sci. Food Agric. 95, 1268–1273.
Jiménez-Cuesta, M.J., Cuquerella, J., Martínez-Jávega, J.M., 1981. Determination of a color index for citrus fruit degreening. Proc. of the International Society of Citriculture 2, 750–753.
Joulain, König, 1998. The Atlas of Spectral Data of Sesquiterpene Hydrocarbons. Verlag, Hamburg.
Kepiro, J.L., Roose, M.L., 2007. Nucellar embryony. In: Khan, I. (Ed.), Citrus Genetics, Breeding and Biotechnology. CABI publishing, Londres, pp. 141–150.
Kijas, J.M.H., Thomas, M.R., Fowler, J.C.S., Roose, M.L., 1997. Integration of trinucleotide microsatellites into a linkage map of Citrus. Theor. Appl. Genet. 94, 701–706.
König, Hochmuth, Joulain, 2001. Terpenoids and Related Constituents of Essential Oils. Library of MassFinder 2.1. University of Hamburg, Institute of Organic Chemistry, Hamburg.
Liu, C., Jiang, D., Cheng, Y., Deng, X., Chen, F., Fang, L., Ma, Z., Xu, J., 2013a. Chemotaxonomic study of Citrus, Poncirus and Fortunella genotypes based on peel oil volatile compounds - deciphering the genetic origin of Mangshanyegan (*Citrus nobilis* Lauriro). PLoS One 8, e58411.
Liu, S.R., Li, W.Y., Long, D., Hu, C.G., Zhang, J.Z., 2013b. Development and characterization of genomic and expressed SSRs in citrus by genome-wide analysis. PLoS One 8, e75149.
Lota, M.L., De Rocca Serra, D., Tomi, F., Casanova, J., 2000. Chemical variability of peel and leaf essential oils of 15 species of mandarins. Biochem. Syst. Ecol. 28, 61–78.
Lota, M.L., De Rocca Serra, D., Tomi, F., Casanova, J., 2001. Chemical variability of peel and leaf essential oils of 15 species of mandarins. Biochem. Syst. Ecol. 29, 77–104.

Lota, M.L., de Rocca Serra, D., Tomi, F., Bessiere, J.M., Casanova, J., 1999. Chemical composition of peel and leaf essential oils of Citrus medica L. and C. limonimedica Lush. Flavour Fragrance J. 14, 161–166.
Lota, M.L., de Rocca Serra, D., Tomi, F., Jacquemond, C., Casanova, J., 2002. Volatile components of peel and leaf oils of lemon and lime species. J. Agric. Food Chem. 50, 796–805.
Luro, F., Costantino, G., Terol, J., Argout, X., Allario, T., Wincker, P., Talon, M., Ollitraul, t P., Morillon, R., 2008. Transferability of the EST-SSRs developed on Nules clementine (*Citrus clementina* Hort ex Tan) to other Citrus species and their effectiveness for genetic mapping. BMC Genomics 9, 287.
Luro, F., Rist, D., Ollitrault, P., 2000. Evaluation of genetic relationships in Citrus genus by means of sequence tagged microsatellites. In: International Symposium on Molecular Markers for Characterizing Genotypes and Identifying Cultivars in Horticulture. Acta Horticulturae, vol. 546. pp. 237–242.
Luro, F., Venturini, N., Costantino, G., Paolini, J., Ollitrault, P., Costa, J., 2012. Genetic and chemical diversity of citron (Citrus medica L.) based on nuclear and cytoplasmic markers and leaf essential oil composition. Phytochemistry 77, 186–196.
Luro, F., Bloquel, E., Tomu, B., Costantino, G., Tur, I., Riolacci, S., Varamo, F., Ollitrault, P., Froelicher, Y., Curk, F., Pailly, O., 2018. The INRA-CIRAD citrus germplasm collection of San Giuliano, Corsica in AGRUMED: archaeology and history of citrus fruit. In: Véronique, Zech-Matterne, Girolamo, Fiorentino (Eds.), The Mediterranean: Acclimatization, Diversifications, Uses. Publications du Centre Jean Bérard, Naples, pp. 243–261.
Luro, F., Laigret, F., Ollitrault, P., Bové, J.M., 1995. DNA amplified fingerprinting (D.A.F.), an useful tool for determination of genetic origin and diversity analysis in Citrus. Hortic. Sci. (Stuttg.) 30, 1063–1067.
Manca Dell'Arca, A., 1780. Agricoltura in Sardegna. Orsino, Napoli.
Mignani, I., Mulas, M., Mantegazza, R., Lovigu, N., Spada, A., Nicolosi, E., Bassi, D., 2004. Caratterizzazione morfologica, biochimica e molecolare di accessioni di “Pompia”, agrume della Sardegna. In: Atti Delle VII Giornate Scientifiche SOI, Castel dell'Ovo, Napoli).
Mignani, I., Mulas, M., Mantegazza, M., Lovigu, N., Spada, A., Nicolosi, E., Bassi, D., 2015. Characterization by molecular markers of “Pompia” a natural Citrus hybrid cultivated in Sardinia. Acta Hortic. (Wagening.) 1065, 165–172.
Moris, G.G., 1837. Flora Sardoa. Tip. Regia, Torino.
National Institute of Standards and Technology, 1999. PC version 1.7 of the NIST/EPA/NIH Mass Spectral Database. Perkin–Elmer Corp., Norwalk.
Nicolosi, E., Deng, Z.N., Gentile, A., La Malfa, S., Continella, G., Tribulato, E., 2000. Citrus phylogeny and genetic origin of important species as investigated by molecular markers. Theor. Appl. Genet. 100, 1155–1166.
Ollitrault, F., Terol, J., Pina, J.A., Navarro, L., Talon, M., Ollitrault, P., 2010. Development of SSR markers from *Citrus clementina* (Rutaceae) BAC end sequences and interspecific transferability in Citrus. Am. J. Bot. 97, 124–129.
Ollitrault, P., Garcia-Lor, A., Terol, J., Curk, F., Ollitrault, F., Talon, M., Navarro, L., 2014. Comparative values of SSRs, SNPs and InDels for citrus genetic diversity analysis. Acta Hortic. (Wagening.) 1065, 457–466.
Ollitrault, P., Jacquemond, C., Dubois, C., Luro, F., 2003. Citrus. In: Hamon, P., Seguin, M., Perrier, X., Glaszmann, X. (Eds.), Genetic Diversity of Cultivated Plants. CIRAD, Montpellier, pp. 193–197.
Oueslati, A., Salhi-Hannachi, A., Luro, F., Vignes, H., Mournet, P., Ollitrault, P., 2016. Genotyping by Sequencing reveals the interspecific C. maxima/C. reticulata admixture along the genomes of modern citrus varieties of mandarins, tangors, tangelos, orangelos and grapefruits. PLoS One 12, e0185618. https://doi.org/10.1371/journal.pone.0185618.
Perrier, X., Flori, A., Bonnot, F., 2003. Methods for data analysis. In: Hamon, Seguin, Perrier, Glaszmann (Eds.), Genetic Diversity of Cultivated Tropical Plants. Science Publishers, Inc. and CIRAD, Montpellier, pp. 31–63.
Petretto, G.L., Sarais, G., Maldini, M.T., Foddai, M., Tirillini, B., Rourke, J.P., Chessa, M., Pintore, G., 2015. *Citrus monstruosa* discrimination among several Citrus species by multivariate analysis of volatiles: a metabolomic approach. J. Food Process. Preserv. 40, 950–957.
Pritchard Lab, S.U., 2014. Structure software. http://pritchardlab.stanford.edu/structure.html.
Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959.
Risso, J.A., Poiteau, A., 1818. Histoire naturelle des oranges, Autod Ed. (Paris).
Rosa, Nieddu, Petretto, Sarais, 2019. Chemical composition and in vitro bioactivity of essential oil obtained from the flavedo of ‘Pompia’, an ancient Sardinian fruit. J. Essent. Oil Res. https://doi.org/10.1080/10412905.2019.1606740.
Snoussi, H., Duval, M.-F., Garcia-Lor, A., Belfalah, Z., Froelicher, Y., Risterucci, A.-M., Perrier, X., Jacquemoud-Collet, J.-P., Navarro, L., Harrabi, M., Ollitrault, P., 2012. Assessment of the genetic diversity of the Tunisian citrus rootstock germplasm. BMC Genet. 13, 16.
Sutour, S., Luro, F., Bradesi, P., Casanova, J., Tomi, F., 2016. Chemical composition of the fruit oils of five Fortunella species grown in the same pedoclimatic conditions in Corsica (France) NPC, vol. 11. pp. 259–262.
Viglietti, G., Galla, G., Porceddu, A., Barcaccia, G., Curk, F., Luro, F., Scarpa, G.M., 2019. Karyological analysis and DNA barcoding of Pompia: a first step toward the identification of its relatives. Plants 8, 83. https://doi.org/10.3390/plants8040083.
Weising, K., Gardner, R.C., 1999. A set of conserved PCR primers for the analysis of simple sequence repeat polymorphisms in chloroplast genomes of dicotyledonous angiosperms. Genome 42, 9–19.
Wu, G.A., Prochnik, S., Jenkins, J., Salse, J., Hellsten, U., Murat, F., Perrier, X., Ruiz, M., Scalabrin, S., Terol, J., Takita, M.A., Labadie, K., Poulain, J., Couloux, A., Jabbari, K., Cattonaro, F., Del Fabbro, C., Pinosio, S., Zuccolo, A., Chapman, J., Grimwood, J., Tadeo, F.R., Estornell, L.H., Muñoz-Sanz, J.V., Ibanez, V., Herrero-Ortega, A., Aleza, P., Pérez-Pérez, J., Ramón, D., Brunel, D., Luro, F., Chen, C., Farmerie, W.G., Desany, B., Kodira, C., Mohiuddin, M., Harkins, T., Fredrikson, K., Burns, P., Lomsadze, A., Borodovsky, M., Reforgiato, G., Freitas-Astúa, J., Quetier, F., Navarro, L., Roose, M., Wincker, P., Schmutz, J., Morgante, M., Machado, M.A., Talon, M., Jaillon, O., Ollitrault, P., Gmitter, F., Rokhsar, D., 2014. Sequencing of diverse Mandarin, pummelo and orange genomes reveals complex history of admixture during citrus domestication. Nat. Biotechnol. 32, 656–662. https://doi.org/10.1038/nbt.2906.
Wu, G.A., Terol, J.F., Ibáñez, V., López-García, A., Pérez-Román, E., Borredá, C., Domingo, C., Tadeo, F., Carbonell-Caballero, J., Alonso, R., Curk, F., Du, D., Ollitrault, P., Roose, M.L., Dopazo, J., Gmitter, F.G., Rokhsar, D.S., Talon, M., 2018. Genomics of the origin and evolution of Citrus. Nature 554 311-136.

Further reading

García-Lor, A., Curk, F., Snoussi-Trifa, H., Morillon, R., Ancillo, G., Luro, F., Navarro, L., Ollitrault, P., 2013. A nuclear phylogenetic analysis: SNPs, indels and SSRs deliver new insights into the relationships in the "true citrus fruit trees" group (Citrinae, Rutaceae) and the origin of cultivated species. Ann. Bot. 111, 1–19. https://doi.org/10.1093/aob/mcs22.